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(54) Title: HUMAN NARCOLEPSY GENE

(57) Abstract: The gene for hypocretin (orexin) receptor 1 (HCRTR1), which is associated with narcolepsy, is disclosed. Also described are methods of diagnosis of narcolepsy, pharmaceutical compositions comprising nucleic acids comprising the HCRTR1 gene, as well as methods of therapy of narcolepsy.

HUMAN NARCOLEPSY GENE

RELATED APPLICATION

This application is a Continuation-in-Part of U.S. Serial No. 09/379,083, filed August 23, 1999, the entire teachings of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Narcolepsy, a disorder which affects approximately 1 in 2,000 individuals, is characterized by daytime sleepiness, sleep fragmentation, and symptoms of abnormal rapid eye movement (REM) sleep that include cataplexy (loss of muscle tone), sleep paralysis, and hypnagogic hallucinations (Aldrich, M.S., *Neurology 42*:34-43 (1992); Siegel, J.M., *Cell 98*:409-412 (1999)). In humans, susceptibility to narcolepsy has been associated with a specific human leukocyte antigen (HLA) alleles, including DQB1*0602 (Mignot, E., *Neurology 50*:S16-22 (1998); Kadotani, H. *et al., Genome Res. 8*:427-434 (1998); Faraco, J. *et al., J. Hered. 90*:129-132 (1999)); however, attempts to verify narcolepsy as an autoimmune disorder have failed (Mignot, E. *et al., Adv. Neuroimmunol. 5*:23-37 (1995); Mignot, E., *Curr. Opin. Pulm. Med. 2*:482-487 (1996)). In a canine model of narcolepsy, the disorder is transmitted as an autosomal recessive trait, *canarc-1* (Foutz, A.S. *et al., Sleep 1*:413-421 91979); Baker, T.L. and Dement, W.C., Brain Mechanisms of Sleep (D.J. McGinty *et al., eds.s,* New York: Raven Press, pp. 199-233 (1985)). The possibility of linkage between *canarc-1* and the canine major histocompatibility complex has been excluded (Mignot, E. *et al., Proc. Natl. Acad. Sci. USA 88*:3475-3478 (1991)).

A mutation in the hypocretin (orexin) receptor 2 gene in canines has been identified in narcolepsy (Lin, L. *et al., Cell 98*:365-376 (1999)); Hypocrexins/orexins (orexin-A and -B) are neuropeptides associated with regulation of food consumption (de Lecea, L., *et al., Proc. Natl. Acad. Sci. USA 95*:322-327 (1998); Sakurai, T. *et al., Cell 92*:573-585 (1998)) as well as other possible functions (Peyron, C. *et al., J. Neurosci. 18*:9996-10015 (1998)). Human cDNA of receptors for orexins have been cloned (Sakurai, T. *et al., Cell 92*:573-585 (1998)), however, full human genes for the orexin receptors have not yet been identified.

Diagnosis of narcolepsy is difficult, as it is necessary to distinguish narcolepsy from other conditions such as chronic fatigue syndrome or other sleep disorders (Ambrogetti, A. and Olson, L.C., *Med. J. Aust. 160*:426-429 (1994); Aldrich, M.S., *Neurology 50*:S2-7 (1998)). Methods of diagnosing narcolepsy based on specific criteria would facilitate identification of the disease, reduce the time and expense associated with diagnosis, and expedite commencement of treatment.

SUMMARY OF THE INVENTION

As described herein, a full gene for the human hypocretin (orexin) receptor 1 (HCRTR1) has been identified. The sequence of the HCRTR1 gene as described herein is shown in Fig. 1 (SEQ ID NO: 1). Accordingly, this invention pertains to an isolated nucleic acid molecule containing the HCRTR1 gene. The invention also relates to DNA constructs comprising the nucleic acid molecules described herein operatively linked to a regulatory sequence, and to recombinant host cells, such as bacterial cells, fungal cells, plant cells, insect cells and mammalian cells, comprising the nucleic acid molecules described herein operatively linked to a regulatory sequence. The invention also pertains to methods of diagnosing narcolepsy in an individual. The methods include detecting the presence of a mutation in the HCRTR1 gene. The invention additionally pertains to pharmaceutical compositions comprising the HCRTR1 nucleic acids of the invention. The invention further pertains to methods of treating narcolepsy, by administering HCRTR1 nucleic acids

of the invention or compositions comprising the HCRTR1 nucleic acids. The methods of the invention allow the accurate diagnosis of narcolepsy and reduce the need for time-consuming and expensive sleep laboratory assessments.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1E depict the sequence of the human orexin receptor 1 gene (SEQ ID NO:1) and the encoded receptor (SEQ ID NO:2).

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a human hypocretin (orexin) receptor 1 (HCRTR1) gene, and the relationship of the gene to narcolepsy. As described herein, Applicants have isolated the HCRTR1 gene. The gene and its products are implicated in the pathogenesis of narcolepsy, as mutations in a closely related receptor, hypocretin (orexin) receptor 2, have been associated with the presence of narcolepsy in a well-established canine model of narcolepsy (Lin, L. *et al., Cell 98*:365-376 (1999)) .

NUCLEIC ACIDS OF THE INVENTION

Accordingly, the invention pertains to an isolated nucleic acid molecule containing the human HCRTR1 gene. The term, "HCRTR1 gene," refers to an isolated genomic nucleic acid molecule that encodes the human hypocretin (orexin) receptor 1. As used herein, the term, "genomic nucleic acid molecule" indicates that the nucleic acid molecule contains introns and exons as are found in genomic DNA (i.e., not cDNA). The nucleic acid molecules can be double-stranded or single-stranded; single stranded nucleic acid molecules can be either the coding (sense) strand or the non-coding (antisense) strand. The nucleic acid molecule can additionally contain a marker sequence, for example, a nucleotide sequence which encodes a polypeptide, to assist in isolation or purification of the polypeptide. Such

sequences include, but are not limited to, those which encode a glutathione-S-transferase (GST) fusion protein and those which encode a hemaglutinin A (HA) peptide marker from influenza. In a preferred embodiment, the nucleic acid molecule has the sequence shown in Figs. 1A-1E (SEQ ID NO:1).

As used herein, an "isolated" or "substantially pure" gene or nucleic acid molecule is intended to mean a gene which is not flanked by nucleotide sequences which normally (in nature) flank the gene (as in other genomic sequences). Thus, an isolated gene can include a gene which is synthesized chemically or by recombinant means. Thus, recombinant DNA contained in a vector are included in the definition of "isolated" as used herein. Also, isolated nucleotide sequences include recombinant DNA molecules in heterologous host cells, as well as partially or substantially purified DNA molecules in solution. Such isolated nucleotide sequences are useful in the manufacture of the encoded protein, as probes for isolating homologous sequences (e.g., from other mammalian species), for gene mapping (e.g., by *in situ* hybridization with chromosomes), or for detecting expression of the HCRTR1 gene in tissue (e.g., human tissue), such as by Northern blot analysis.

The present invention also encompasses variations of the nucleic acid sequences of the invention. Such variations can be naturally-occurring, such as in the case of allelic variation, or non-naturally-occurring, such as those induced by various mutagens and mutagenic processes. Intended variations include, but are not limited to, addition, deletion and substitution of one or more nucleotides which can result in conservative or non-conservative amino acid changes, including additions and deletions. Preferably, the nucleotide or amino acid variations are silent or conserved; that is, they do not alter the characteristics or activity of the hypocretin (orexin) receptor 1.

Other alterations of the nucleic acid molecules of the invention can include, for example, labeling, methylation, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates), charged linkages (e.g., phosphorothioates, phosphorodithioates), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen),

chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids). Also included are synthetic molecules that mimic nucleic acid molecules in the ability to bind to a designated sequences via hydrogen bonding and other chemical interactions. Such molecules include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The invention also relates to fragments of the isolated nucleic acid molecules described herein. The term "fragment" is intended to encompass a portion of a nucleic acid sequence described herein which is from at least about 25 contiguous nucleotides to at least about 50 contiguous nucleotides or longer in length. One or more introns can also be present. Such fragments are useful as probes, e.g., for diagnostic methods, as described below and also as primers or probes. Particularly preferred primers and probes selectively hybridize to a nucleic acid molecule containing the HCRTR1 gene described herein.

The invention also pertains to nucleic acid molecules which hybridize under high stringency hybridization conditions, such as for selective hybridization, to a nucleotide sequence described herein (e.g., nucleic acid molecules which specifically hybridize to a nucleic acid containing the HCRTR1 gene described herein). Hybridization probes are oligonucleotides which bind in a base-specific manner to a complementary strand of nucleic acid. Suitable probes include polypeptide nucleic acids, as described in (Nielsen *et al.*, *Science* 254, 1497-1500 (1991)).

Such nucleic acid molecules can be detected and/or isolated by specific hybridization (e.g., under high stringency conditions). "Stringency conditions" for hybridization is a term of art which refers to the incubation and wash conditions, e.g., conditions of temperature and buffer concentration, which permit hybridization of a particular nucleic acid to a second nucleic acid; the first nucleic acid may be perfectly (i.e., 100%) complementary to the second, or the first and second may share some degree of complementarity which is less than perfect (e.g., 60%, 75%, 85%, 95%). For example, certain high stringency conditions can be used which distinguish perfectly complementary nucleic acids from those of less complementarity.

"High stringency conditions", "moderate stringency conditions" and "low stringency conditions" for nucleic acid hybridizations are explained on pages 2.10.1-2.10.16 and pages 6.3.1-6 in *Current Protocols in Molecular Biology* (Ausubel, F.M. *et al.*, "*Current Protocols in Molecular Biology*", John Wiley & Sons, (1998)) the teachings of which are hereby incorporated by reference. The exact conditions which determine the stringency of hybridization depend not only on ionic strength (e.g., 0.2XSSC, 0.1XSSC), temperature (e.g., room temperature, 42°C, 68°C) and the concentration of destabilizing agents such as formamide or denaturing agents such as SDS, but also on factors such as the length of the nucleic acid sequence, base composition, percent mismatch between hybridizing sequences and the frequency of occurrence of subsets of that sequence within other non-identical sequences. Thus, high, moderate or low stringency conditions can be determined empirically. By varying hybridization conditions from a level of stringency at which no hybridization occurs to a level at which hybridization is first observed, conditions which will allow a given sequence to hybridize (e.g., selectively) with the most similar sequences in the sample can be determined.

Exemplary conditions are described in Krause, M.H. and S.A. Aaronson, *Methods in Enzymology, 200*:546-556 (1991). Also, in, Ausubel, *et al.*, "*Current Protocols in Molecular Biology*", John Wiley & Sons, (1998), which describes the determination of washing conditions for moderate or low stringency conditions. Washing is the step in which conditions are usually set so as to determine a minimum level of complementarity of the hybrids. Generally, starting from the lowest temperature at which only homologous hybridization occurs, each °C by which the final wash temperature is reduced (holding SSC concentration constant) allows an increase by 1% in the maximum extent of mismatching among the sequences that hybridize. Generally, doubling the concentration of SSC results in an increase in T_m of ~17°C. Using these guidelines, the washing temperature can be determined empirically for high, moderate or low stringency, depending on the level of mismatch sought.

For example, a low stringency wash can comprise washing in a solution containing 0.2XSSC/0.1% SDS for 10 min at room temperature; a moderate

stringency wash can comprise washing in a prewarmed solution (42°C) solution containing 0.2XSSC/0.1% SDS for 15 min at 42°C; and a high stringency wash can comprise washing in prewarmed (68°C) solution containing 0.1XSSC/0.1%SDS for 15 min at 68°C. Furthermore, washes can be performed repeatedly or sequentially to obtain a desired result as known in the art. Equivalent conditions can be determined by varying one or more of the parameters given as an example, as known in the art, while maintaining a similar degree of identity or similarity between the target nucleic acid molecule and the primer or probe used.

Hybridizable nucleic acid molecules are useful as probes and primers, e.g., for diagnostic applications, as described below. As used herein, the term "primer" refers to a single-stranded oligonucleotide which acts as a point of initiation of template-directed DNA synthesis under appropriate conditions (*e.g.*, in the presence of four different nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer, but typically ranges from 15 to 30 nucleotides. Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template, but must be sufficiently complementary to hybridize with a template. The term "primer site" refers to the area of the target DNA to which a primer hybridizes. The term "primer pair" refers to a set of primers including a 5' (upstream) primer that hybridizes with the 5' end of the DNA sequence to be amplified and a 3' (downstream) primer that hybridizes with the complement of the 3' end of the sequence to be amplified.

The invention also pertains to nucleotide sequences which have a substantial identity with the nucleotide sequences described herein; particularly preferred are nucleotide sequences which have at least about 70%, and more preferably at least about 80% identity, and even more preferably at least about 90% identity, with nucleotide sequences described herein. Particularly preferred in this instance are nucleotide sequences encoding hypocretin (orexin) receptor 1.

To determine the percent identity of two nucleotide sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first nucleotide sequence). The nucleotides at corresponding nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions x 100).

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin *et al.* (*Proc. Natl. Acad. Sci. USA*, *90*:5873-5877 (1993)). Such an algorithm is incorporated into the NBLAST program which can be used to identify sequences having the desired identity to nucleotide sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (*Nucleic Acids Res, 25*:3389-3402 (1997)). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. In one embodiment, parameters for sequence comparison can be set at W=12. Parameters can also be varied (e.g., W=5 or W=20). The value "W" determines how many continuous nucleotides must be identical for the program to identify two sequences as containing regions of identity.

The invention also provides expression vectors containing a nucleic acid comprising the HCRTR1 gene, operatively linked to at least one regulatory sequence. Many such vectors are commercially available, and other suitable vectors can be readily prepared by the skilled artisan. "Operatively linked" is intended to mean that the nucleic acid sequence is linked to a regulatory sequence in a manner which allows expression of the nucleic acid sequence. Regulatory sequences are art-recognized and are selected to produce a hypocretin (orexin) receptor 1. Accordingly, the term "regulatory sequence" includes promoters, enhancers, and

other expression control elements such as those described in Goeddel, *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, CA (1990). For example, the native regulatory sequences or regulatory sequences native to the transformed host cell can be employed. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the receptor desired to be expressed. For instance, the gene of the present invention can be expressed by ligating the gene into a vector suitable for expression in either prokaryotic cells, eukaryotic cells or both (see, for example, Broach, *et al.*, *Experimental Manipulation of Gene Expression*, ed. M. Inouye (Academic Press, 1983) p. 83; *Molecular Cloning: A Laboratory Manual*, 2nd Ed., ed. Sambrook *et al.* (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17). Typically, expression constructs will contain one or more selectable markers, including, but not limited to, the gene that encodes dihydrofolate reductase and the genes that confer resistance to neomycin, tetracycline, ampicillin, chloramphenicol, kanamycin and streptomycin resistance. Vectors can also include, for example, an autonomously replicating sequence (ARS), expression control sequences, ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, secretion signals and mRNA stabilizing sequences.

Prokaryotic and eukaryotic host cells transformed by the described vectors are also provided by this invention. For instance, cells which can be transformed with the vectors of the present invention include, but are not limited to, bacterial cells such as *E. coli* (e.g., *E. coli* K12 strains), *Streptomyces*, *Pseudomonas*, *Serratia marcescens* and *Salmonella typhimurium*, insect cells (baculovirus), including *Drosophila*, fungal cells, such as yeast cells, plant cells and mammalian cells, such as thymocytes, Chinese hamster ovary cells (CHO), and COS cells. The host cells can be transformed by the described vectors by various methods (e.g., electroporation, transfection using calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection, infection where the vector is an infectious agent such as a retroviral genome, and other methods), depending on the type of cellular host.

The nucleic acid molecules of the present invention can be produced, for example, by replication in a suitable host cell, as described above. Alternatively, the nucleic acid molecules can also be produced by chemical synthesis.

The nucleotide sequences of the nucleic acid molecules described herein (e.g., a nucleic acid molecule comprising SEQ ID NO:1) can be amplified by methods known in the art. For example, this can be accomplished by e.g., PCR. *See generally PCR Technology: Principles and Applications for DNA Amplification* (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); *PCR Protocols: A Guide to Methods and Applications* (eds. Innis, *et al.*, Academic Press, San Diego, CA, 1990); Mattila *et al.*, *Nucleic Acids Res.* 19, 4967 (1991); Eckert *et al.*, *PCR Methods and Applications* 1, 17 (1991); *PCR* (eds. McPherson *et al.*, IRL Press, Oxford); and U.S. Patent 4,683,202.

Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, *Genomics* 4, 560 (1989), Landegren *et al.*, *Science* 241, 1077 (1988), transcription amplification (Kwoh *et al.*, *Proc. Natl. Acad. Sci. USA* 86, 1173 (1989)), and self-sustained sequence replication (Guatelli *et al.*, *Proc. Nat. Acad. Sci. USA,* 87, 1874 (1990)) and nucleic acid based sequence amplification (NASBA). The latter two amplification methods involve isothermal reactions based on isothermal transcription, which produce both single stranded RNA (ssRNA) and double stranded DNA (dsDNA) as the amplification products in a ratio of about 30 or 100 to 1, respectively.

The amplified DNA can be radiolabeled and used as a probe for screening a library or other suitable vector to identify homologous nucleotide sequences. Corresponding clones can be isolated, DNA can be obtained following *in vivo* excision, and the cloned insert can be sequenced in either or both orientations by art recognized methods, to identify the correct reading frame encoding a protein of the appropriate molecular weight. For example, the direct analysis of the nucleotide sequence of homologous nucleic acid molecules of the present invention can be accomplished using either the dideoxy chain termination method or the Maxam - Gilbert method (see Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd Ed., CSHP, New York 1989); Zyskind *et al.*, *Recombinant DNA Laboratory*

Manual, (Acad. Press, 1988)). Using these or similar methods, the protein(s) and the DNA encoding the protein can be isolated, sequenced and further characterized.

METHODS OF DIAGNOSIS

The nucleic acids and the proteins described above can be used to detect, in an individual, a mutation in the HCRTR1 gene that is associated with narcolepsy. In one embodiment of the invention, diagnosis of narcolepsy is made by detecting a mutation in the HCRTR1 gene. The mutation can be the insertion or deletion of a single nucleotide, or of more than one nucleotide, resulting in a frame shift mutation; the change of at least one nucleotide, resulting in a change in the encoded amino acid; the change of at least one nucleotide, resulting in the generation of a premature stop codon; the deletion of several nucleotides, resulting in a deletion of one or more amino acids encoded by the nucleotides; the insertion of one or several nucleotides, such as by unequal recombination or gene conversion, resulting in an interruption of the coding sequence of the gene; duplication of all or a part of the gene; transposition of all or a part of the gene; or rearrangement of all or a part of the gene. More than one such mutation may be present in a single gene. Such sequence changes cause a mutation in the receptor encoded by the HCRTR1 gene. For example, if the mutation is a frame shift mutation, the frame shift can result in a change in the encoded amino acids, and/or can result in the generation of a premature stop codon, causing generation of a truncated receptor. Alternatively, a mutation associated with narcolepsy can be a synonymous mutation in one or more nucleotides (i.e., a mutation that does not result in a change in the receptor encoded by the HCRTR1 gene). Such a polymorphism may alter splicing sites, affect the stability or transport of mRNA, or otherwise affect the transcription or translation of the gene. A HCRTR1 gene that has any of the mutations described above is referred to herein as a "mutant gene." It is likely that a mutation in the HCRTR1 gene is associated with narcolepsy in humans because of the association between a mutation in the HCRTR1 gene and narcolepsy in dogs (Lin, L. *et al., Cell 98*:365-376 (1999), the entire teachings of which are incorporated herein by reference). In a preferred embodiment, the mutation in the HCRTR1 gene is to a deletion mutation, for

example, a deletion that corresponds to the deletions found in the hypocretin (orexin) receptor 2 in narcoleptic dogs as described by Lin *et al.*, *supra* (e.g., a deletion of one or more exons, such as a deletion of the fourth exon, that can be caused by insertion of one or more nucleotides upstream of the splice site of the exon, or a deletion of exon 6, that can be caused by a G to A transition in the splice junction consensus sequence). In another preferred embodiment, the mutation in the HCRTR1 gene is mutation that effects a "knockout" of the entire gene, such as deletion of the first exon as described by Chemelli, R.M. *et al,* (*Cell 98*:437-451 (1999), the entire teachings of which are incorporated herein).

In a first method of diagnosing narcolepsy, hybridization methods, such as Southern analysis, are used (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, including all supplements through 1999). For example, a test sample of genomic DNA, RNA, or cDNA, is obtained from an individual suspected of having (or carrying a defect for) narcolepsy (the "test individual"). The individual can be an adult, child, or fetus. The test sample can be from any source which contains genomic DNA, such as a blood sample, sample of amniotic fluid, sample of cerebrospinal fluid, or tissue sample from skin, muscle, placenta, gastrointestinal tract or other organs. A test sample of DNA from fetal cells or tissue can be obtained by appropriate methods, such as by amniocentesis or chorionic villus sampling. The DNA, RNA, or cDNA sample is then examined to determine whether a mutation in the HCRTR1 gene is present. The presence of the mutation can be indicated by hybridization of the gene in the test sample to a nucleic acid probe. A "nucleic acid probe", as used herein, can be a DNA probe or an RNA probe; the nucleic acid probe contains at least one mutation in the HCRTR1 gene. The probe can be one of the nucleic acid molecules described above (e.g., the gene, a vector comprising the gene, etc.)

To diagnose narcolepsy by hybridization, a hybridization sample is formed by contacting the test sample containing a HCRTR1 gene, with at least one nucleic acid probe. The hybridization sample is maintained under conditions which are sufficient to allow specific hybridization of the nucleic acid probe to the HCRTR1 gene. "Specific hybridization", as used herein, indicates exact hybridization (e.g.,

with no mismatches). Specific hybridization can be performed under high stringency conditions or moderate stringency conditions, for example, as described above. In a particularly preferred embodiment, the hybridization conditions for specific hybridization are high stringency.

Specific hybridization, if present, is then detected using standard methods. If specific hybridization occurs between the nucleic acid probe and the HCRTR1 gene in the test sample, then the HCRTR1 gene has the mutation that is present in the nucleic acid probe. More than one nucleic acid probe can also be used concurrently in this method. Specific hybridization of any one of the nucleic acid probes is indicative of a mutation in the HCRTR1 gene, and is therefore diagnostic for narcolepsy.

In another hybridization method, Northern analysis (see Current Protocols in Molecular Biology, Ausubel, F. *et al.*, eds., John Wiley & Sons, *supra*) is used to identify the presence of a mutation associated with narcolepsy. For Northern analysis, a test sample of RNA is obtained from the individual by appropriate means. Specific hybridization of a nucleic acid probe, as described above, to RNA from the individual is indicative of a mutation in the HCRTR1 gene, and is therefore diagnostic for narcolepsy

For representative examples of use of nucleic acid probes, see, for example, U.S. Patents No. 5,288,611 and 4,851,330. Alternatively, a peptide nucleic acid (PNA) probe can be used instead of a nucleic acid probe in the hybridization methods described above. PNA is a DNA mimic having a peptide-like, inorganic backbone, such as N-(2-aminoethyl)glycine units, with an organic base (A, G, C, T or U) attached to the glycine nitrogen via a methylene carbonyl linker (see, for example, Nielsen, P.E. *et al., Bioconjugate Chemistry*, 1994, 5, American Chemical Society, p. 1 (1994). The PNA probe can be designed to specifically hybridize to a gene having a polymorphism associated with autoimmune disease. Hybridization of the PNA probe to the HCRTR1 gene is diagnostic for narcolepsy..

In another method of the invention, mutation analysis by restriction digestion can be used to detect mutant genes, or genes containing polymorphisms, if the mutation or polymorphism in the gene results in the creation or elimination of a

restriction site. A test sample containing genomic DNA is obtained from the individual. Polymerase chain reaction (PCR) can be used to amplify the HCRTR1 gene (and, if necessary, the flanking sequences) in the test sample of genomic DNA from the test individual. RFLP analysis is conducted as described *(see* Current Protocols in Molecular Biology, supra). The digestion pattern of the relevant DNA fragment indicates the presence or absence of the mutation in the HCRTR1 gene, and therefore indicates the presence or absence of narcolepsy.

Sequence analysis can also be used to detect specific mutations in the HCRTR1 gene. A test sample of DNA is obtained from the test individual. PCR can be used to amplify the gene, and/or its flanking sequences. The sequence of the HCRTR1 gene, or a fragment of the gene is determined, using standard methods. The sequence of the gene (or gene fragment) is compared with the nucleic acid sequence of the gene, as described above. The presence of a mutation in the HCRTR1 gene indicates that the individual has narcolepsy.

Allele-specific oligonucleotides can also be used to detect the presence of a mutation in the HCRTR1 gene, through the use of dot-blot hybridization of amplified proteins with allele-specific oligonucleotide (ASO) probes (see, for example, Saiki, R. *et al.*, (1986), *Nature (London) 324*:163-166). An "allele-specific oligonucleotide" (also referred to herein as an "allele-specific oligonucleotide probe") is an oligonucleotide of approximately 10-50 base pairs, preferably approximately 15-30 base pairs, that specifically hybridizes to the HCRTR1 gene, and that contains a mutation associated with narcolepsy. An allele-specific oligonucleotide probe that is specific for particular mutation in the HCRTR1 gene can be prepared, using standard methods (see Current Protocols in Molecular Biology, supra). To identify mutations in the gene that are associated with narcolepsy, a test sample of DNA is obtained from the individual. PCR can be used to amplify all or a fragment of the HCRTR1 gene, and its flanking sequences. The DNA containing the amplified HCRTR1 gene (or fragment of the gene) is dot-blotted, using standard methods (see Current Protocols in Molecular Biology, supra), and the blot is contacted with the oligonucleotide probe. The presence of specific hybridization of the probe to the amplified HCRTR1 gene is then detected. Specific

hybridization of an allele-specific oligonucleotide probe to DNA from the individual is indicative of a mutation in the HCRTR1 gene, and is therefore indicative of narcolepsy.

Other methods of nucleic acid analysis can be used to detect mutations in the HCRTR1 gene, for the diagnosis of narcolepsy. Representative methods include direct manual sequencing; automated fluorescent sequencing; single-stranded conformation polymorphism assays (SSCA); clamped denaturing gel electrophoresis (CDGE) heteoduplex analysis; chemical mismatch cleavage (CMC); RNase protection assays; use of proteins which recognize nucleotide mismatches, such as *E. coli* mutS protein; allele-specific PCR, and other methods.

PHARMACEUTICAL COMPOSITIONS

The present invention also pertains to pharmaceutical compositions comprising nucleic acids described herein, particularly nucleic acids containing the HCRTR1 gene described herein. For instance, a nucleotide or nucleic acid construct (vector) comprising a nucleotide of the present invention can be formulated with a physiologically acceptable carrier or excipient to prepare a pharmaceutical composition. The carrier and composition can be sterile. The formulation should suit the mode of administration.

Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions (e.g., NaCl), saline, buffered saline, alcohols, glycerol, ethanol, gum arabic, vegetable oils, benzyl alcohols, polyethylene glycols, gelatin, carbohydrates such as lactose, amylose or starch, dextrose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid esters, hydroxymethylcellulose, polyvinyl pyrolidone, etc., as well as combinations thereof. The pharmaceutical preparations can, if desired, be mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, flavoring and/or aromatic substances and the like which do not deleteriously react with the active compounds.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid

solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, polyvinyl pyrollidone, sodium saccharine, cellulose, magnesium carbonate, etc.

Methods of introduction of these compositions include, but are not limited to, intradermal, intramuscular, intraperitoneal, intraocular, intravenous, subcutaneous, oral and intranasal. Other suitable methods of introduction can also include gene therapy (as described below), rechargeable or biodegradable devices, particle acceleration devises ("gene guns") and slow release polymeric devices. The pharmaceutical compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

The composition can be formulated in accordance with the routine procedures as a pharmaceutical composition adapted for administration to human beings. For example, compositions for intravenous administration typically are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

For topical application, nonsprayable forms, viscous to semi-solid or solid forms comprising a carrier compatible with topical application and having a dynamic viscosity preferably greater than water, can be employed. Suitable formulations include but are not limited to solutions, suspensions, emulsions, creams, ointments, powders, enemas, lotions, sols, liniments, salves, aerosols, etc., which are, if desired,

sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers or salts for influencing osmotic pressure, etc. The agent may be incorporated into a cosmetic formulation. For topical application, also suitable are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant, e.g., pressurized air.

Agents described herein can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The agents are administered in a therapeutically effective amount. The amount of agents which will be therapeutically effective in the treatment of narcolepsy can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use of sale for human administration. The pack or kit can be labeled with information regarding mode of administration, sequence of drug administration (e.g., separately, sequentially or concurrently), or the like. The pack or kit may also include means for reminding the patient to take the therapy. The

pack or kit can be a single unit dosage of the combination therapy or it can be a plurality of unit dosages. In particular, the agents can be separated, mixed together in any combination, present in a single vial or tablet. Agents assembled in a blister pack or other dispensing means is preferred. For the purpose of this invention, unit dosage is intended to mean a dosage that is dependent on the individual pharmacodynamics of each agent and administered in FDA approved dosages in standard time courses.

METHODS OF THERAPY

The present invention also pertains to methods of therapy for narcolepsy, utilizing the pharmaceutical compositions comprising nucleic acids, as described herein. The therapy is designed to replace/supplement activity of the hypocretin(orexin) receptor 1 in an individual, such as by administering a nucleic acid comprising the HCRTR1 gene or a derivative or active fragment thereof. In one embodiment of the invention, a nucleic acid of the invention is used in the treatment of narcolepsy. The term, "treatment" as used herein, refers not only to ameliorating symptoms associated with the disease, but also preventing or delaying the onset of the disease, and also lessening the severity or frequency of symptoms of the disease. In this embodiment, a nucleic acid of the invention (e.g., the HCRTR1 gene (SEQ ID NO:1)) can be used, either alone or in a pharmaceutical composition as described above. For example, the HCRTR1 gene, either by itself or included within a vector, can be introduced into cells (either *in vitro* or *in vivo*) such that the cells produce native HCRTR1 receptor. If necessary, cells that have been transformed with the gene or can be introduced (or re-introduced) into an individual affected with the disease. Thus, cells which, in nature, lack native HCRTR1 expression and activity, or have mutant HCRTR1 expression and activity, can be engineered to express HCRTR1 receptors (or, for example, an active fragment of the HCRTR1 receptor). In a preferred embodiment, nucleic acid comprising the HCRTR1 gene, can be introduced into an expression vector, such as a viral vector, and the vector can be introduced into appropriate cells which lack native HCRTR1 expression in an animal. In such methods, a cell population can be engineered to inducibly or

constitutively express active HCRTR1 receptor. Other gene transfer systems, including viral and nonviral transfer systems, can be used. Alternatively, nonviral gene transfer methods, such as calcium phosphate coprecipitation, mechanical techniques (e.g., microinjection); membrane fusion-mediated transfer via liposomes; or direct DNA uptake, can also be used.

The nucleic acids and/or vectors are administered in a therapeutically effective amount (i.e., an amount that is sufficient to treat the disease, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or also lessening the severity or frequency of symptoms of the disease). The amount which will be therapeutically effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of a practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

The following Examples are offered for the purpose of illustrating the present invention and are not to be construed to limit the scope of this invention. The teachings of all references cited herein are hereby incorporated herein by reference.

EXAMPLES

EXAMPLE 1 Identification of the Human Narcolepsy Gene

A human BAC library (RPCI11 human male BAC library; see Osoegawa, K. *et al., Genomics 52*:1-8 (1998)) was used. Seventeen primers, designed from the mRNA sequence of the HCRTR1 receptor, were employed to identify clones of interest. They are set forth in Table 1.

TABLE 1 Primers Used for Hybridization

Number	Name	Primer Sequence	SEQ ID NO:
1	HCRTR1-1-F	TCAGGAAGTTTGAGGCTGAGA	3
2	HCRTR1-1-R	ATCCTAGGCTCTACAGAGGGA	4
3	HCRTR1-2-F	GAAGATGAGTTTCTCCGCTATC	5
4	HCRTR1-2-R	GATGAGGACCCACTCATACTG	6
5	HCRTR1-3-F	ACATGAGGACAGTCACCAACTA	7
6	HCRTR1-3-R	CAGATAGCAGTCACCAGAACG	8
7	HCRTR1-4-F	ACATCACTGAGTCCTGGCTGT	9
8	HCRTR1-4-R	ATGAAGCTGAGAGTTAGCACTG	10
9	HCRTR1-5-F	CTATTGTTCAAGAGCACAGCC	11
10	HCRTR1-5-R	CATCACAGACTGAGAAGAGCC	12
11	HCRTR1-6-R	CTTCACTTCAGCCAGGAAGG	13
12	HCRTR1-7-F	AATGTCCTTAAGAGGGTGTTCG	14
13	HCRTR1-7-R	GAAGTTGTAGATGATGGGGTTG	15
14	HCRTR1-8-F	CACAAGTCCTTGTCCTTGCAG	16
15	HCRTR1-8-R	CACCACATGCTCAGAGATTTTG	17
16	HCRTR1-9-F	CCTACCCCTCATGGAAAGAC	18
17	HCRTR1-9-R	ATCCAGAGTCACACAGGCAGA	19

Initial Study with Large Membranes

Four out of 5 membranes having the whole BAC library, containing a total of approximately 160,000 BAC clones representing an approximately 10-fold coverage of the human genome, were used in hybridization studies with these primers. Hybridization was performed with a pool of all 17 primers described in Table 1.

5' End Labeling for Big Membranes

Oligonucleotides were labeled at the 5' end before hybridization, using fresh [γ^{32}P]ATP (6000 Ci/mmole; 10 μCi/μl). Briefly, a labeling mixture was made of

DNA (8 pmol/μl) (10.0 μl of the primer pool), 10X buffer (12.0 μl), T4 PNK (10 u/μl) (6.0 μl), [γ^{32}P]ATP (30.0 μl, or 600 μCi), and water (62.0 μl) for a final volume of 120 μl. 20 μl of labeling mixture was used per 10 ml rapid hybridization reaction. Incubation of the labeling mixture was for 2 hours at 37°C, followed by transfer to ice, spinning down, and mixing with the rapid hybridization solution. The membranes were prehybridized at 42°C before the labeling mix was added. Sixty μl of the labeling mix was added to each of 2 big bottles containing 2 membranes and 30 ml of rapid hybridization solution.

Hybridization and Washing

The membranes were hybridized at 42°C overnight. After overnight, membranes were washed with 6x SSC, 0.1% SDS at room temperature; washed with 6x SSC, 0.1% SDS at 55°C in a shaking waterbath, repeated until the radioactivity of membranes was lower than 6k using 1x sensitivity; and washed with 6x SSC to remove the SDS. The washed membranes were put in a cassette for overnight exposure at -80°C with a MR single emulsion film. Positive clones were identified and gridded on small membranes.

Study of Positive Clones with Small Membranes

After growing the positively-identified clones on several small membranes (to get several copies of membranes containing the same clones), and washing the membranes, hybridization was performed using pairs of primers, instead of a total pool of primers as before. The total number of hybridizations was nine, using different primers against identical copies of membranes containing all positive clones from the first hybridization. The primer pairs are set forth in Table 2; primer numbers indicate the primers shown in Table 1.

TABLE 2 Primer Pairs Used for Hybridization

Reaction number	Primers Used
1	1 and 2
2	3 and 4
3	5 and 6
4	7 and 8
5	9 and 10
6	11
7	12 and 13
8	14 and 15
9	16 and 17

5' End Labeling for Small Membranes

Oligonucleotides were labeled at the 5' end before hybridization, using fresh [γ^{32}P]ATP (5000 Ci/mmole; 10 μCi/μl). Briefly, a labeling mixture was made of DNA (8 pmol/μl) (1.5 μl), 10X buffer (2.0 μl), T4 PNK (10 u/μl) (1.0 μl), [γ^{32}P]ATP (3.0 μl), and water (12.5 μl) for a final volume of 20 μl. Incubation of the labeling mixture was for 2.5 hours at 37°C, followed by transfer to ice, spinning down, and mixing with the rapid hybridization solution. Membranes were pre-wetted in 6X SSC, rolled in a pipette, and excess liquid drained prior to placing the membrane in the tube. Fifty ml Falcon (polypropylene) tubes were used as container for the hybridization. The membranes were prehybridized at 42°C before 20 μl of labeling mix was added to each tube.

Hybridization and Washing

The membranes were hybridized at 42°C overnight. After overnight, membranes were washed as described above. Four clones which were positive for primers designed using the 5' and 3' end of the mRNA were identified. Clone 333N1 was used to characterize the gene.

Sequencing of Narcolepsy Gene in Clone 33N1

Shotgun sequencing, supplemented with sequencing of PCR products amplified from 333N1, was used to obtain the gene sequence.

Shotgun Sequencing

Preparation of DNA Samples

BAC DNA was isolated using the Plasmix kit from TALENT-VH Bio Limited. Thirty μg of isolated DNA was fragmented by nebulization: a nebulizer (IPI Medical Products, Inc., no. 4207) was modified by removing the plastic cylinder drip ring, cutting off the outer rim of the cylinder, inverting it and placing it back into the nebulizer; the large hole in the top cover (where the mouth piece was attached) was sealed with a plastic stopper; the small hole was connected to a 1/4 inch length of Tycon tubing (connected to a compressed air source). A DNA sample was prepared containing 30 μg DNA, 10 X TM buffer (200 μl), sterile glycerol (1 ml), and sterile dd water (q.s.) for a total volume of 2 ml. The DNA sample was nebulized in an ice-water bath for 2 minutes and 40 seconds (pressure bar reading 0.5). The sample was then briefly centrifuged at 2500 rpm to collect the DNA; the entire unit was placed in the rotor bucked of a table top centrifuge (Beckman GPR tabletop centrifuge) fitted with pieces of Styrofoam to cushion the nebulizer. The sample was then distributed into four 1.5 ml microcentrifuge tubes and ethanol precipitated. The Dried DNA pellet was resuspended in 35 μl of 1X TM buffer prior to proceeding with fragment end-repair.

Fragment End Repair, Size Selection and Phosphorylation

The DNA was resuspended in 27 μl of 1X TM buffer. The following materials were added: 10 X kinase buffer (5 μl), 10 mM rATP (5 μl), 0.25 mM dNTPs (7 μl), T4 polynucleotide kinase (1 μl (3 U/μl)), Klenow DNA polymerase (2 μl (5 U/μl)), T4 DNA polymerase (1 μl (3 U/μl)), for a total volume of 48 μl. The mixture was incubated at 37°C for 30 minutes, and then 5 μl of agarose gel loading dye was added. The mixture was then applied to separate wells of a 1% low

melting temperature agarose gel and electrophoresed for 30-60 minutes at 100-120 mA. The DNA was then eluted from each sample lane, extracted from the agarose using Ultrafree-DA columns (Millipore) and then cleaned with Microcon-100 columns (Amicon), precipitated in ethanol, and resuspended in 10 µl of 10:0.1 TE buffer.

Ligation

EcoRV-linearized, CIAP-dephosphorylated Bluescript vector was used as a cloning vector. The following reagents were combined in a microcentrifuge tube, and incubated overnight at 4°C: DNA fragments (100-1000 ng), cloning vector (2 µl (10 ng/µl)), 10X ligation buffer (1 µl), T4 DNA ligase (NEB 202L) (1 µl (400 U/µl)), sterile dd water (q.s.), for a total of 10 µl.

Transformation of Ligated Products

The ligation products were diluted 1:5 with dd water and used to transform electrocompetent TOP 10F cells (Invitrogen) using GenePulser II (Biorad; voltage, 2.5 W, resistance 100 ohm). Transformants were plated on LB plates with 50 µl of 4% X-GAL and 50 µl of 4% IPTG, and ampicillin. Transformants were grown overnight at 37°C, white colonies were picked, grown in a culture of 3 ml LB liquid media plus 200 µg/µl ampicillin for 16-20 hours with shaking. DNA was isolated from the liquid cultures using Autogen 740 Automatic Plasmid Isolation System.

Cycle Sequencing of Isolated Plasmid DNA

Isolated plasmids were then sequenced using the M13 primers: M13-forward (SEQ ID NO:20) TGTAAAACGACGGCCAG; and M13-reverse (SEQ ID NO:21) CAGGAAACAGCTATGAC. For the sequencing reaction, 2.5 µl plasmid template was mixed with 4 µl Big Dye Ready reaction mix (ABI), 1 µl of 8 pM M13 primer, and 2.5 µl dd water. For cycle sequencing, 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60 °C for 4 minutes were performed, followed by holding at 4°C. The cycle sequencing reaction products were cleaned by spinning through Sephadex G-50 columns. The eluted cycle sequencing products were then dissolved in 3 µl

formamide/dye and 1.5 µl of sample was loaded on ABI 377 automated sequencers. The data was analyzed using Phred and Phrap (Ewing, B. *et al., Genome Res. 8*:175-185 (1998); Ewing, B. and Green, P., *Genome Res. 8*:186-194 (1998)), and viewed in Consed viewer (Gordon, D. *et al., Genome Res. 8(3)*:195-202 (1998)).

PCR Product Sequencing

In order to supplement the sequencing described above, PCR products of BAC 33N1 were also sequenced. Primers used for the hybridizations, as described above, were used to amplify regions of 333N1 by long PCR using GeneAmpXL PCR kit (Perkin Elmer). The sequence for HCRTR1-6F is as follows: TCTTTATTGTCACCTACCTGGC (SEQ ID NO:22).

TABLE 3 PCR Product Sequencing Pairs

Primer Pair Used for PCR	Primers Used for Cycle Sequencing of PCR Product
HCRTR1-1F/HCRTR1-4R	HCRTR1-1F, 2F, 3F, 4F, 1R, 2R, 3R, 4R
HCRTR1-1F/HCRTR1-6R	HCRTR1-1F, 2F, 3F, 5F, 6F, 1R, 2R, 3R
HCRTR1-4F/HCRTR1-7R	HCRTR1-4F, 5F, 6F, 7F, 4R, 5R, 6R, 7R
HCRTR1-6F/HCRTR1-8R	HCRTR1-6F, 6R, 7R, 8R
HCRTR1-7F/HCRTR1-9R	HCRTR1-7F, 8F, 7R, 8R, 9R

The PCR products were prepared for cycle sequencing by incubation at 37°C for 15 minutes and then at 87°C for 15 minutes to destroy the enzymes. The PCR products were then subject to cycle sequencing as described above, with the same procedures for sequencing gel runs and sequence analysis.

Analysis of Gene Structure

The *hcrtr-1* gene maps to chromosome 1p33. A total of 9,785 base pairs of contiguous sequence was generated for 333N1 which contained all of the *hcrtr-1* gene. Comparison of the cDNA sequence of *hcrtr-1* (Accession number

GI:4557636) and the genomic sequences generated allowed deduction of the intron/exon organization of the gene. The gene contains 7 exons which cover 8,077 bp. There were two discrepancies in the base sequence of the published mRNA sequence for hcrtr-1 (Accession number GI:4557636) and the genomic sequence described herein. The first discrepancy was located in base number 264 in the mRNA sequence and this site represents a SNP. The second discrepancy could either be a polymorphism or an error in the generation of the mRNA sequence.

The splice junctions of the *hcrtr-1* gene are set forth in Table 4, and the intron sizes are set forth in Table 5. Exon sequences are represented in uppercase and introns in lowercase. All splice sites conform to the consensus GT-AG rule. SEQ ID NOs are given in the column immediately following each site.

Table 4 Splice Junctions of *hcrtr-1*

	Splice Donor Site	SEQ ID.	Splice Acceptor Site	SEQ ID
Hcrtr-1 exon1-2	CGCTGGgtaggt	23	ctgcagTCTGCC	24
Hcrtr-1 exon2-3	CTACAGgtgagc	25	gggcagGCTGTG	26
Hcrtr-1 exon3-4	GGGCAGgtaatg	27	ctgcagATGACC	28
Hcrtr-1 exon4-5	CGCCAGgtgagg	29	ggacagATCCCC	30
Hcrtr-1 exon5-6	TAAGAGgtgaga	31	gcctagGGTGTT	32
Hcrtr-1 exon6-7	TCAGTGgtgagc	33	accaagGCAAAT	34

Table 5 Intron Sizes of *hcrtr-1*

Intron	Nucleotides
Intron 1	140
Intron 2	1,132
Intron 3	390
Intron 4	1,929
Intron 5	1,248
Intron 6	1,671

The exons do not clearly respect the domain structure of this seven membrane domain G protein linked receptor. Five of the transmembrane regions are by themselves within one exon, two of the transmebrane segments are broken up by introns, and two transmembrane segments fall within the same exon. A survey done one year ago on mammalian G-protein coupled receptors (GPCRs) sequences in GenBank revealed that over 90% of GPCRs genes were intronless in their open reading frame (ORF) (Gentles, A.J. and Karlin, S., *Trends Genet. 15*:47-49 (1999)). Comparison of the intron/exon boundaries of *hcrtr-1* and the genes coding for their most related GPCRs based on sequence similarity showed that the location of the intron/exons boundaries with respect to the transmembrane domains is only partially conserved among the receptors (Sakurai, T. *et al.*, *Cell 92*:573-585 (1998)).

Computer analysis of sequence data

Analysis of the genomic sequence of *hcrtr-1* using the program RepeatMasker (http://ftp.genome.washington.edu/cgi-bin/RepeatMasker) showed that the sequence containing the *hcrtr-1* genomic sequence is 25.28% repeat sequences and the GC content is 55.64%.

The sequences of the genes were analyzed using the program GeneMiner (Óskarsson and Pálsson, unpublished), which combines the results of 5 exon prediction programs; FGENE (Solovyev, V. and Salamov, A., *Ismb 5*:294-302 (1997)), Genscan (Burge, C. and Karlin, S., *J. Mol. Biol. 268*:78-94 (1997)),

HMMgene (Krogh, A., *Ismb 5*:179-186 (1997)), MZEF (Zhang, M.Q., *Proc. Natl. Acad. Sci. USA 94*:565-8 (1997)) and Xpound (Thomas, A. and Skolnick, M.H., *IMA J. Math Appl. Med. Biol. 11*:149-160 (1994)). For *hcrtr-1* all the exon predicting programs predicted the 3′end of exon 1 and 5′end of exon 7 correctly and at least three programs out of the five predicted each of the internal exons exactly correct.

The promoter sequences of the genes have not yet been characterized. The Promoter Prediction by Neural Network (http://www.fruitfly.org/seq_tools/promoter.html) predicted promoters that are at least 720 bp upstream of the 5′UTR of *hcrtr-1*, indicating that either a part of the 5′UTR is missing in the published mRNA sequence or the real promoters are not detected by the program.

Analysis of Population for Polymorphisms

Each exon and its flanking intronic sequences of the *hcrtr-1* gene was analyzed in nucleic acid samples from 47 patients and 75 control individuals. The patient population consisted of patients of Icelandic and US origin. The control population consisted of Icelandic controls, CEPH (**C**entre d'**E**tude du **P**olymorphisme **H**umain) individuals from Utah and France, and US samples of various ethnic origins. The African-American/Caucasian ratios were similar between patients and controls. All narcoleptic subjects complained of excessive daytime sleepiness (EDS). Approximately 66% of the patients had cataplexy, 24% did not and 10% did not have attainable records of cataplexy status. Narcoleptic subjects without cataplexy had Multiple Sleep Latency Tests showing mean sleep latencies of less than 10 minutes and REM sleep in at least 2 naps. Subjects did not take any drugs affecting sleep for at least 10 days before their sleep studies.

To analyze the nucleic acids, DNA from patient and control blood samples were isolated by the method of Kunkel (Kunkel, L.M. *et al., Proc. Natl. Acad. Sci. USA 74*:1245-9 (1977)). Briefly, white blood cells were lysed in a sucrose lysis buffer, and proteinase K treated; the DNA was then extracted using phenol-chloroform/isoamylalcohol and then ethanol precipitated. Patient samples that were received in the form of nuclei pelleted through sucrose buffer were resusupended in

lysis buffer (100 mM NaCl2; 10 mM TrisHCl, pH 8; 25 mM EDTA pH 8; 0.5% sodium dodecyl sulfate; 0.1 mg/ml proteinase K) at 55°C for 4-6 hours followed by classical phenol-chloroform extraction and ethanol precipitation (Sambrook, J. *et al., Molecular Cloning, A Laboratory Manual* (1989)). Samples were incubated at 55°C after isolation for the inactivation of DNAse to prevent the degradation of DNA. Concentration of the isolated DNA was determined by spectrophotometric analysis at 260 nm (Sambrook *et al.*,using GeneQuant (PharmaciaBiotech), and samples diluted with sterile distilled water to a 20 ng/μl working solution.

Primers were designed from intronic sequences flanking the exons of the hypocretin receptor-1 (*hcrtr-1*), using either primer design programs available at primer3 at the Whitehead Institute (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi) or primers for the worldwide web (http://williamstone.com/primers/javascript/). The primers are shown in Table 6.

Table 6 Primers Used to Amplify Nucleic Acid Fragments for Analysis of *hcrtr-1* Gene

EXON	#	Primer Sequence	Sense /Anti-sense	External/ Nested	SEQ ID NO.
1	1	TCTGTCTTCCTCCACCAATTTCATGAC	S	E	35
1	2	CATCCTAGTTCTGCCTAATCAGCGAGC	A	E	36
1	3	TTCCTCCACCAATTTCATGACTGTGAG	S	N	37
1	4	GTTCTGCCTAATCAGCGAGCCCTCCTC	A	N	38
1	5	TAGAGCCTAGGATGCCCCTCTGCTGCA	S	N	39
1	6	CTCATCTTCATAGTCTGGAGGCACAGG	A	N	40
2	7	AGGGCTCGCTGATTAGGCAGAACTAGG	S	E	41
2	8	CACTGACCTGTCTGAAAGGCAAGTCTG	A	E	42
2	9	GCTGATTAGGCAGAACTAGGATGGGTG	S	N	43
2	10	ACTGAGACCTATGCAAGGCCGTGGGTT	A	N	44
3	11	CTGCCGTCAGCCTCCTCACTCACCTAC	S	E	45
3	12	CACTTTGGAAGGGACCCTGCTAGATGC	A	E	46
3	13	TCAGCCTCCTCACTCACCTACTCTCAC	S	N	47
3	14	TGGAAGGGACCCTGCTAGATGCCTGTC	A	N	48
4	15	CTCCTAGGCCTTGCTTTGGCCGTAGTC	S	E	49
4	16	GTGCTGATGGCAGAGGACTGCCTACTC	A	E	50
4	17	CCGTAGTCAGGACAGGGTGGCATTGCT	S	N	51
4	18	ATGGCAGAGGACTGCCTACTCAGTTAG	A	N	52
5	19	CCGGGGTCCAGCCTGGAGTA	S	E	53
5	20	CCCAAGCAAGGAGAGCTCCTTCC	A	E	54
5	21	GTCCAGCCTGGAGTAGGCCCCAC	S	N	55
5	22	AGGAGAGCTCCTTCCCCAACCTC	A	N	56
6	23	GTGGACAGAAGTGGGCAGTAGGAACTC	S	E	57
6	24	ATCACTGTGCCCACAGCAGGTACATCC	A	E	58
6	25	AGAAGTGGGCAGTAGGAACTCTTGCAC	S	N	59
6	26	ACAGCAGGTACATCCTCACCCACCATC	A	N	60

EXON	#	Primer Sequence	Sense /Anti-sense	External/ Nested	SEQ ID NO.
1	1	TCTGTCTTCCTCCACCAATTTCATGAC	S	E	35
7	27	GCTCTCCCTCCCAGCTCTATCCCT	S	E	61
7	28	TCAGCATGCTTAATCCTCACATCAACC	A	E	62
7	29	TCCCAGCTCTATCCCTCCCTCCCT	S	N	63
7	30	AGCATGCTTAATCCTCACATCAACCCT	A	N	64
7	31	AAGGCCCCTTCCTGCTGCATCTGTCTC	S	E	65
7	32	TCACAGTTTACAAGGAGCTTTCCACTTGC	A	E	66
7	33	GCCCCTTCCTGCTGCATCTGTCTCC	S	N	67
7	34	TTACAAGGAGCTTTCCACTTGCTTCAG	A	N	68

PCR reactions were done in 20 µl reactions using 40 ng genomic DNA, 0.2 mM solution of the four dNTPs, 0.35 µM of each primer (TAGCopenhagen), 2.5 mM MgCl2 (Perkin Elmer), 1x PCR Buffer (Perkin Elmer) and 0.5 U Amplitaq gold (Perkin Elmer). The primers were used to amplify the fragments by PCR cycling at 95°C for 12 min and subsequently 30 cycles of 95°C for 30 sec, 55-62°C for 30 sec and 72°C for 1 min. 5-15% DMSO was used in the PCR reactions for some primer pairs; DMSO is an organic solvent which facilitates the denaturing of DNA and can therefore be of help when amplifying GC-rich fragments of genomic DNA. The PCR products were prepared for cycle sequencing by incubation with Shrimp alkaline phosphatase (Amersham) and exonuclease I (Amersham) at 37°C for 15 min. After the inactivation of the enzymes the products were subject to cycle sequencing using BigDye Ready Reaction mix (Perkin Elmer) and subsequently run on ABI Prism 377 Automated DNA sequencers. The raw data were basecalled and sequences assembled using the Phred and Phrap software, respectively (Ewing, B. *et al., Genome Res. 8*:175-185 (1998); Ewing, B. and Green, P., *Genome Res. 8*:186-194 (1998)). The Consed viewer was used to analyze the sequences (Gordon, D. *et al., Genome Res. 8(3)*:195-202 (1998)). Allele calling was done using TrueAllele

and editing was done using DeCODE-GT (Palsson, B. *et al., Genome Res. 9*:1002-1012 (1999)).

A total of 12 single nucleotide polymorphisms were identified, 10 in exons and 2 in an intronic sequence near an exon. The polymporphisms are shown in Table 7. The base number is according to the mRNA sequence (Accession number GI:4557636). For those polymorphisms marked with an asterisk (*), the polymorphism is located 3' of the corresponding exons; the numbers indicate the distance into the introns.

Table 7 Single Nucleotide Polymorphisms in *hcrtr-1*

Location	cDNA base #	Nucleic Acid Change
5′ UTR	31	C-A
Exon 1	264	C-T
Intron1	+21*	G-T
Exon 2	354	C-T
Exon 2	390	G-A
Exon 2	363	C-T
Exon 3	652	G-A
Exon 5	989	G-A
Exon 5	995	G-A
Intron 6	+6*	C-T
Exon 7	1375	G-A
3′ UTR	1460	G-A

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. Isolated nucleic acid molecule comprising the nucleic acid having SEQ ID NO:1.

2. A DNA construct comprising the isolated nucleic acid molecule of Claim 1 operatively linked to a regulatory sequence.

3. A recombinant host cell comprising the isolated nucleic acid molecule of Claim 1 operatively linked to a regulatory sequence.

4. A pharmaceutical composition comprising a nucleic acid comprising the isolated nucleic acid molecule of Claim 1.

5. Isolated nucleic acid molecule comprising the nucleic acid having SEQ ID NO:1 with one or more of the nucleic acid changes shown in Table 7.

6. A method of diagnosing narcolepsy in an individual, comprising detecting a mutation in the gene encoding hypocretin (orexin) receptor 1, wherein the presence of the mutation in the gene is indicative of narcolepsy.

7. A method of treating narcolepsy in an individual, comprising administering to the individual an isolated nucleic acid of Claim 1 in a therapeutically effective amount.

```
LOCUS           _____     9785 bp   DNA        PRI       12-NOV-1999
DEFINITION      Human hypocretin (orexin) receptor 1 (HCRTR1) gene, complete cds,complete sequence.
ACCESSION       _____
NID             _____
VERSION         _____
KEYWORDS        .
SOURCE          human.
  ORGANISM      Homo sapiens
                Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
                Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE       1  (bases 1-9785)
AUTHORS         _____
  TITLE         Direct Submission
  JOURNAL               Submitted (_____) deCode Genetics, Inc., Lynghals 1,
                        IS-110  Reykjavik, Iceland.
FEATURES                Location/Qualifiers
     source             1.. 9785
                        /organism="Homo sapiens"
                        /db_xref="taxon : 9606"
                        /chromosome="1"
                        /map="1p33"
                        /clone="BAC 333N1"
     gene               1117..9190
                        /gene="HCRTR1"
                        /note="OX1R"
                        /db_xref="LocusID:3061"
                        /db_xref="MIM:602392"
     exon               1117..1468
                        /gene="HCRTR1"
                        /number=1
     CDS                join(1270..1468, 1609..1787, 2920..3163, 3554..3669, 5599..5825, 7074..7195,
                        8867..9190)
                        /gene="HCRTR1"
                        /note="HCRTR1 exons defined by comparison to mRNA sequence (NM_001525)"
                        /codon_start=1
                        /product="HCRTR1/orexin receptor 1"
                        /db_xref="LocusID:3061"
                        /db_xref="MIM:602392"
                        /protein_id="NP_001516.1"
                        /db_xref="PID:g4557637"
                        /db_xref="GI:4557637"
                        /translation="MEPSATPGAQMGVPPGSREPSPVPPDYEDEFLRYLWRDYLYPKQ
                        YEWVLIAAYVAVFVVALVGNTLVCLAVWRNHHMRTVTNYFIVNLSLADVLVTAICLPA
                        SLLVDITESWLFGHALCKVIPYLQAVSVSVAVLTLSFIALDRWYAICHPLLFKSTARR
                        ARGSILGIWAVSLAIMVPQAAVMECSSVLPELANRTRLFSVCDERWADDLYPKIYHSC
                        FFIVTYLAPLGLMAMAYFQIFRKLWGRQIPGTTSALVRNWKRPSDQLGDLEQGLSGEP
                        QPRARAFLAEVKQMRARRKTAKMLMVVLLVFALCYLPISVLNVLKRVFGMFRQASDRE
                        AVYACFTFSHWLVYANSAANPIIYNFLSGKFREQFKAAFSCCLPGLGPCGSLKAPSPR
                        SSASHKSLSLQSRCSISKISEHVVLTSVTTVLP
     exon               1609..1787
                        /gene="HCRTR1"
                        /number=2
```

FIG. 1A

exon	2920..3163
	/gene="HCRTR1"
	/number=3
exon	3554..3669
	/gene="HCRTR1"
	/number=4
exon	5599..5825
	/gene="HCRTR1"
	/number=5
exon	7074..7195
	/gene="HCRTR1"
	/number=6
exon	8867..9190
	/gene="HCRTR1"
	/number=7

BASE COUNT 2212 a 2759 c 2685 g 2129 t

AAAGGACCCGCGGCGAGGGATGGGAGGAGCCAAGAGTCTCGGGGGGTAACCTGGGTGCTG
GGAGACTGGCTCCTCGGCCAGCGCTGCTCTCCTCTAGGCAGGCTCCGAGTGCCCTCGCTC
CCCCGCGCCTTCCCGGAGCCCCGCCAGCCCCCGAGGTGCAGGAAGGTCCCGCGGACGGAG
CGGGCCTGCCGGCGTTCAGTGGGGTATGAAGGCGTCCCCTCCCCTTCCCCAGGGGTCTCC
AGGGATCCGCAACCCTCCGGCACCTGGCCGGGGTCGTCCTAGCCCAGCCGGGGGAAGGAG
GGGCTGAGTGCGGGAGGAGGGGAGGGGCGGGGAGCTGGGCTGGCTGGATTTATGAATGGA
GAGCGACCCGCGCGCCGGAACAGCGGCTCCTGGCGGCCGTCGGGGAGCGTCGCGGCCCTG
GGGACCCAAAGGGGCCTCTTAGGGGGCCTGGATGCTCCCCTTGCTCGCAAGGGGTCGTCA
GTCCCTCCGCGCACCACCCCCACTGTGTGTGTGTTGTATGTGTGCGTGTCCGCGAGTGCC
CACCGGAGGGTCTGGCAGGTATGGCGGGTGGGGCTTGGGTCTCTAACACCTCCCTTGGCC
CGTTTTCCCAACCCAAAGTTAAAGCCTCTGAACTGGCTCAAGAAATATTTGCAATCGGGA
TGGCTTCTCCTCCCAAATCTACGGTGTTTGGTGGGTTCGAACAGACCTGGCTTAAGAGCT
TTGTGACCTTGAGCAAGAGACTCAATCTCTCTGAGCTTCGGTCTCATCTGCAAAGCAGGG
TACCCTAATAATGGTAACCGGAAACGTCCCCGAAACTACCTTCTCGTACCAGGTTCTTGG
TGAAGCACTTGGCACGCATCGGAGCTCATTACTCCTCATCGTGGTCCTGTAAGGTATGTA
GGGCTGTCACCCCATTAGACAGATGGGGAAACCAAGGCTGAAAGAGGCCAGGTAAGCTAC
CCAAGGCAACTGGTGTGGAATTGGGATGCAACCCAGGTCTGTCTTCCTCCACCAATTTCA
TGACTGTGAGAATTAAGAGGGAACTTATACGCAAAGCGCCTGGCACAATCCCTAATGTTT
CCTTCCTTCTCTCTTTTCCCACTCCCTCCTTTCCTTCCTCCCTTCAGGAAGTTTGAGGCT
GAGACCCGAAAAGACCTGGGTGCAAGCCTCCAGGCACCCTGAAGGGAGTGGGCTGAGGGC
TGGCCCAAGCTCCCTCCTCTCCCTCTGTAGAGCCTAGGATGCCCCTCTGCTGCAGCGGCT
CCTGAGCTCATGGAGCCCTCAGCCACCCCAGGGGCCCAGATGGGGGTCCCCCCTGGCAGC
AGAGAGCCGTCCCCTGTGCCTCCAGACTATGAAGATGAGTTTCTCCGCTATCTGTGGCGC
GATTATCTGTACCCAAAACAGTATGAGTGGGTCCTCATCGCAGCCTATGTGGCTGTGTTC
GTCGTGGCCCTGGTGGGCAACACGCTGGGTAGGTCCAGGGCTTGCCCGGCAGTGCTGCCG
GCTTTCCCTGGGGATTGAAGGGGGTTGTGTGGGAGGAGGGCTCGCTGATTAGGCAGAACT
AGGATGGGTGTGGCTCTGCCACCAGCTTCACCTCGCTGCACCCTGCAGTCTGCCTGGCCG
TGTGGCGGAACCACCACATGAGGACAGTCACCAACTACTTCATTGTCAACCTGTCCCTGG
CTGACGTTCTGGTGACTGCTATCTGCCTGCCGGCCAGCCTGCTGGTGGACATCACTGAGT
CCTGGCTGTTCGGCCATGCCCTCTGCAAGGTCATCCCCTATCTACAGGTGAGCTCTGCCC
AGGCACCCCTCACCACTCCTTGTCACGCCTGTAAAAAACCCACGGCCTTGCATAGGTCTC
AGTGACCCCCAGACTTGCCTTTCAGACAGGTCAGTGGCTCATGACCCCTGAAGTGTCATC
CTCTGCTGCTAGCAAGGGCAAGCCACCAGATCAGACACTCGAGGACACAGACACAACCCC
ACACACTCACAGAGATCCCCTCCTGGTCACAGCCACAGACATATACATAGACACGTGTGG
ACATGTATAGTCACCTCCAGGTACACAGGCACACAGTCAAGGAGAGAGGCAACAGCCCAC
AGTGACACATACACGACACCCTAGGCCTGCTCCCCAATCCCAAAGGGGCAGACGTGAGGG
GCCTGATGGAAACAGCCGTCTCCTCTCCCTCCTGCACTGGCCAGGAAAGACCCCAGTGGT

FIG. 1B

GGAAACCAGGATGTCCGGATGGGGTTAGTGGGGTGGAAGGAAGGCTTCTCTCAGTTTGTA
TCCTGTGATCCACTTCCTGCACCCCAGAGGGCAGGGGGCACCCCTAGAGGCAATGCCCAC
ACACCTCTGACCCAGACTCATCTCTGCCTCCCAGAATGAGGGCTTTTTCCTAACAGCCTG
GGGAAGGGATGGCATTCCATGGCAGAGATAAATGCCTCTTGGATTTCCCACTATTTTGAG
GCTCCCCACTCAACTGGTTAACTCTGGTGACCCTGAGCATAAAGACAGATGGATGAGGGA
ATTCTGTGCCTCAGTTTCCTCATCTGTAACAGGGGGGCAAGAGCGCTTTGTGGGATTGTC
ATGAGGATGATGAGAACAGTGCCCAGCACATAGTAAGTTACGTAGGTGCAAGTTATTATT
CACCGGAGGGGTGCACCTACCATGTGCCAGGTCTAAAGCTGGACATTGTTTGTACATGAT
TTCACTTATTCGCACAAGAATCTTGCCAGGTAGATGGTATATTCCCATTCTGTAGACGAG
GCTCAGAGAGGGTGAGTGACTTGCCCACATTTACACAGCCAGTAAGTGGTGGAGTCAGGA
TTTGCACTGCCCTGCACCTGCCGTCAGCCTCCTCACTCACCTACTCTCACATCGCTGGGT
GGCCCCCAAAATGACCGACGTTGTGTCCCCGTGGGGCAGGCTGTGTCCGTGTCAGTGGCA
GTGCTAACTCTCAGCTTCATCGCCCTGGACCGCTGGTATGCCATCTGCCACCCACTATTG
TTCAAGAGCACAGCCCGGCGGGCCCGTGGCTCCATCCTGGGCATCTGGGCTGTGTCGCTG
GCCATCATGGTGCCCCAGGCTGCAGTCATGGAATGCAGCAGTGTGCTGCCTGAGCTAGCC
AACCGCACACGGCTCTTCTCAGTCTGTGATGAACGCTGGGCAGGTAATGGTGGAAGCCTC
AAGCAGGCATCCCCTCAGGTGGGCACTTTGGGAGCACGTACCCCTAGGACAGGCATCTAG
CAGGGTCCCTTCCAAAGTGGGAAATCCCAGAGCAGGTATTTCCCTAGGGGACACCCTAGA
CTGGCTCCTACCAGGGATACTCCCAGGGTGGGTGCCTCCCCTCATGTAGACATCTGCTCT
AGTGTAGATGTCCTTCCAGGAGGGACAACCCAAGTTGGACAACTCCAGGGTCTCTGTCTG
TCATGGTGGCTGTATGGGGTCCAGCTGCTCCTAGGCCTTGCTTTGGCCGTAGTCAGGACA
GGGTGGCATTGCTAACCAGGGCAGGGTGGGGCTCACGGATTGGGCCTGACTCTGCATCTC
TTGACCCCTGCAGATGACCTCTATCCCAAGATCTACCACAGTTGCTTCTTTATTGTCACC
TACCTGGCCCCACTGGGCCTCATGGCCATGGCCTATTTCCAGATATTCCGCAAGCTCTGG
GGCCGCCAGGTGAGGCCCACTCTGGGCAGGGGCTAGGCCAGTCACTGTGTGGGCTGGGGG
TGGGAGGGCTACTGGTCTAACTGAGTAGGCAGTCCTCTGCCATCAGCACATGCCATCTTG
GCTGCAACCAAAGAGAGGGGAAGCCCAGAGACACGTCAAACTCAAGGCCAAAAGCACCAG
TGGCTACCCTGGAATGGAATAGTAACACGTCCTTCTATTAGTGGTTGGCGTTTATTGAAG
TATCCACTCCCAGATAATCTTGCATCCTCTTAGCCACCATATATTACCCACATTAAATAT
ATGAGAAAACCGAGACCCAGAAGATCAACATAACTTCCCCCAAACCACTCAGCTAGTGAG
TAGATCAGGAACTAAAGCCCAGATCTGTGAGCTCCCACTGCTCAGTTTAGTACCACTGCA
ACAATAATAATAGCAACTCCGTGGTGCTTGCCAAATTAGGCACTTTGCATCCAATGTCTT
AACAACTATCTAACAAAAGAAGCAACATTACCCACGTCACAAATGCCAAATAAGGGCAAC
CAACTTGCCAGATTCAACAGCAGCAGAGCCTTCTGGTTCCAGGGCCTGTCTTCTTTCCTG
CATTACAGACTGACCCACGGTGGGTTTCTTAGGTTTTTGGGGGGCAGGGGTGGTCAGAGG
CCCTTGGCCTAGCGAGTGGGAGTCCTGGATTGGCGTCTGGGCGGTGAGAAAAGGCAGGCC
AGAACATGACCAGGCTCAGGAAGGGACTCTCACACTTGGGGATGTCACCTACATTCCACA
GGAAGTACTGGCTTGCACCCAGGCCATGCCGGGCAGCGGATGGGGACACGGACTGGCTGT
GACCCAGGTCCTGCCTTGGAGGAGCACCCAGTCCAGTAGGACCCTTCCTGACTGGCCAGC
CCTGTAGTCCACCAACACTCATCATCTGCTCCCCACAGACCCCCCAGCCAAGCAGGACAC
AGGCACGATCCTCCTCATTTGACAGATATGAAAGCAAGGCTTAGAAAGGAAAATGAGGTG
GCTAAGGTCACATAGCTCACGAATGGCTGAGCTGGCTCTAGACCCGCGTTTCCAGAACTC
CAGCCCCATGCCCCTCTGTGGTGGGTGATTTGAGTGTCCGGTGGCAGGAGAGGCTTCTCC
AGGAGCCCAGAACCACCCCAGGCTTATGGGCACTGGCCCAGGCCATTCGATGCTGCCCAC
CTGCTCACCCCTTGCCCAGGCCTCCTCATAGTCTGGTATGATCCAGGGGAGGCACAACTC
ACCCCCACCCCTACCCTCAAAGATAGTGTTGGAGATTTAGGGAGGATGGATGGGCAGTTG
ACAGGATGTGGCCTGGGGTCTTGTCAAGGTTCCCCACCTCTTTGAGTCTTAGTTGCCTCA
TCTATACCTAAGGACCAATAATATCTTTCCACAAGGCGTGTTGTAGAGGGTTTCACAAAG
AGCTAATGGAAAATGAAAGTCTAGGCTGGGCGCAGTGGCTCACACCTGTATTCCCAGCAC
TTTGGGAGGCTGAGGCAGGCGGATCACCTAAGGTCAGGAGTTCAAGACCAGCCTGGCCAA
CGTGGTGAAACCCCATCTCTACTAAAAATACAAAACTTAGCCCGGTGTGGTGGCGCACAC
CTGTAATCCCAGCTACTCGGGAGGCGAGATTGAAGAGAGCCAAGATTGCACCATTGCACT
CCAGCTTAGGTGACAAGAGTGAAATGCCATCTCAAAAAAAAAAAAAAAAGAAAAGAAAAGA
AAATGAAAGTCTATCGTTCACTCTCAAGTCCAGAGTGTTAGTCTATCATAAACATTAGAT
TCCTTCCTCTTGCAAGGGTTTTATCCTTTTGCCCATCTCCACCCTGCCCGGGGTCCAGCC

FIG. 1C

TGGAGTAGGCCCCACAAAAGGCAACCACCCTCCCAAGGTGCTGTACCCACCACTGCTGTC
TCTATGTGTGCTGGACAGATCCCCGGCACCACCTCAGCACTGGTGCGGAACTGGAAGCGC
CCCTCAGACCAGCTGGGGGACCTGGAGCAGGGCCTGAGTGGAGAGCCCCAGCCCCGGGCC
CGCGCCTTCCTGGCTGAAGTGAAGCAGATGCGTGCACGGAGGAAGACAGCCAAGATGCTG
ATGGTGGTGCTGCTGGTCTTCGCCCTCTGCTACCTGCCCATCAGCGTCCTCAATGTCCTT
AAGAGGTGAGAGCACGGGGTATGGTTGGGGTGGGGAGAAGTTTGAGGTTGGGGAAGGAGC
TCTCCTTGCTTGGGAGAAAGACCTGGCTCCACCCCTTCTCCACTATGTGATCTTGGGCAG
GCCATTTCTCTTCTCTGAGCCTCCATCTCCTAGGGCTATCGTGAAAATTCACGCATTCAT
TCACTTAATCATCACATTTTAGGGGGCTGGAAATACAATGAACAAGTGCATAAGACAGAC
AAAGTCCCTGCCTTCATGGAGGCTGCATTCTAGCAGGAGAGAAGGGAAGTAAATAGAAGA
ATCAATGTATATTATAATGTCAGGCAGTGATAACTGCTGGGAAGAAAAATAAAATAGGAC
AGAGAGTGACAATGATAAGGGTTGGTGGGTTTTTGCTTTTGCTTTAGATACAATGGTTTA
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AAGGAGGGAGGATCGCTTGAGGCCAGGAGTTCAAGATCAGCCCGGGCAACATAATGAGAC
TTCGTCTCTACTAAAATTCAAAAAATTAGCCAGCCATGGTGGCATGTGCCTGTAGTTCTA
GCTACACAGACTGAGGTGGAAGAATAGCTTGAGCCCAGGAGGTTGAGGCTGCAGCGAACC
ATGATTGCACCACTGCACTCCAGCCTGGGTGACACAGCTGTCTCAAAAAAAAAAAAAAAA
AAAAAAAAGCCTTTCCAAGGAAATGACATTTGAGCAGAGACTTGAAGGAAGTGAGAGAGC
TAACCATGCACGTGTCTGTAGGGACAGCCAAAGAGGGTCGCAGGGCGCTGGGGAGAGAAT
GCAGGCTATTGGACAGAAGACAGTTTCACTTTGAGATTGTGCTTGGCCACTTCCTGGTTG
TGTGATCTTCGGCATGTCACTTTACTTCTCTGAGCCTCAGTTTCCTTAATGGAAAAATGG
ATGATGTCTATGATTCATCATGTTGCTGTGAGGATGGATGAGAAAGTGGATGGGAAGCCC
CAGGGGATCCGATGGCCAGGAGGCTAGAGATGCCCATCACGGTGCTTGATACCCTCCATG
CTTGAGAACCCCAAACCCTGGCCAAGACCTCAGGTACAGAAGGCCAGGAAACGTGGACAG
AAGTGGGCAGTAGGAACTCTTGCACTTTACAGCTCAGGTTCTGTGAGCAGCACTCCCCCA
GTACATGCATACGCAGCTACCCCATTTCTGACGCTCCTCCACCCTGGGCCTAGGGTGTTC
GGGATGTTCCGCCAAGCCAGTGACCGCGAAGCTGTCTACGCCTGCTTCACCTTCTCCCAC
TGGCTGGTGTACGCCAACAGCGCTGCCAACCCCATCATCTACAACTTCCTCAGTGGTGAG
CAGGCTGGGGATGCAAAATGACTGAGGGTGGCCAACAGTCCACATGACAAGTCTCCCCAT
CCCCAAGCCAGGGCCCAAATAAAGGATGGTGGGTGAGGATGTACCTGCTGTGGGCACAGT
GATCCTGCTCTGGGAGGACCCACCCCAAGCGGCCCTGGCCTGAGTGGGAGACGGGCCACA
CTCCCTACAGTGGCTGGCACCCAGGATCCAGTTTTGCAGATTCTGCAGACCAGTGAGTGA
GTGGAAGGGCAGGGGCTAGGCCAGCTCACCCCCAACTCCCACCCTGGGTGCAGGCACAGC
AAGACCTCCAATCAGCTCAGGCAGAGGAGTCCATCCTCCCCGGAGGGAGTCAGACCTGTG
GGAGGAGGGCCCTGGAGCCCCTGCCCGAGGAAGGATTGCACAGTCCAGGTGTCAGGGCTA
AAGTAGGGTCACTCTGAGAGACAAGCCAGGCCCAGGGAAGGGCTTCGCCGGCTCAGCTAG
ACACACTGGCAGAGTGACCGGAATCTCAGGGGTTGTCCCCTCTGGAAGTCTTCCTCCCCT
GCCACCCCCACTCCCACTCCAGGCCTCTCCTCTCTGCTGTCCCACAGTGCCCACCCCCTC
CCTCTACCTCCCAGTCTCAGGGTGGTAATGGCTCTGAGGCTGAGCTCAGCAGAAGTCTGA
CTCACCAGCCCTCTGACTTTGGGAATAGACTTCTAAAGAACAGGTCCAGATGACTGTTGA
AGCCTGGACAGAAATAATCTTTGAGGAACTATTAAAAGGTTAAAGAAAGGATCAGGAGTC
AATAGTATAACCCTCATTGAGACTCAAGAATTACTCAACAAGGCTGGCTGCGGGTTTCCA
GGTCAGAAAAGAGAATAGATGATGAGCTGTGTGGGGAGGGGAGGGCAGACAGACTTACTG
ACACATATGCCTTTGTTTGGCCTATGTTTACTGAGCACCTACTATGTGCTTGACCCTGTG
CTGGGCACCAGAGAGGCTGGCAGCCTAATGACACATGATCAAAGGGGCTTCAGCCTGACA
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CCTCCCCCTTGAGAAGGGCTTTGGAATTAGAATTGGGTTCAGCTTCTGGCTGGGTGGACT
TGGGCAAGCCACTGTACCTCTGTGCATCTCATCTGTGAAGTGAGGATAAAGGACTCCAGC
CTTTCAGGGTGCTGGGATGCTCTGGCGGACAGAGGCTGAGGCGCCCAGCACAGCGTGACT
GCCAAATGCAAAAGGGCTGCTGCTGCCGTCATTTTCATCATCAAAGGGCAGAGAGGACAC
AAGCCTCGCAACAGATAGTGACCCCCACGTACACACCAAGGAGAGCAGAGGTGACCTGAG
GCCCCCGAGCCAGACACCACGTTTTGAGTCAGCCTCCGAGCCAGAGCACAGTCAAGGAAT
CAGATGGCAATTGCGTCTCTCCTTGGGAACCCGCTCCAGGGCTTCTGTCCTCTCTCTCTG
GCGGTGCCGAGGTTGCCTCAGGGCTCTCCCTCCCAGCTCTATCCCTCCCTCCCTCCCCGC
CCCCTCATAGGCAGCTTGGCTGGAGCTGCGTGGGTGTCCCTGGGCTCAAGGCCCCTTCCT

FIG. 1D

GCTGCATCTGTCTCCTTATGGCTGTGTCTTTTGTCTCCCAACCAAGGCAAATTCCGGGAG
CAGTTTAAGGCTGCCTTCTCCTGCTGCCTGCCTGGCCTGGGTCCCTGCGGCTCTCTGAAG
GCCCCTAGTCCCCGCTCCTCTGCCAGCCACAAGTCCTTGTCCTTGCAGAGCCGATGCTCC
ATCTCCAAAATCTCTGAGCATGTGGTGCTCACCAGCGTCACCACAGTGCTGCCCTGAGCG
AGGGCTGCCCTGGAGGCTCCGGCTCGGGGGATCTGCCCCTACCCCTCATGGAAAGACAGC
TGGATGTGGTGAAAGGCTGTGGCTTCAGTCCTGGGTTTCTGCCTGTGTGACTCTGGATAA
GTCACTTCCTCTGTCTGAGCTTGTGTCCCCTAAGCAGGGTTGATGTGAGGATTAAGCATG
CTGAAGCAAGTGGAAAGCTCCTTGTAAACTGTGAAGTGTTGTGGACATGATTATTGTTGT
ACTTCTCTCATTTGGCCATACCCCACAGTATAATCTGTCCCCATCCTCCTTCCAGAGCTT
GGTCATCCTCCTAAAGACCCCTTTCCTACCCAATTACAGGCCTTCCCTGGAGTCTGCTCT
AAAGGTCCCAACAGGCATTTCCATCTTGTTCCATGGCTCCCTGAAGCCCAGGGCTGCACT
TGGCCAGCTGTTCTGATGCCTGTGTGAACTAATCTGGGCCCAGCCTTTCTCCAGCGGGCC
ACGAGCACAGCCCCACCCTAACCAGGTGCCAAGGGCACACACCACAGACCCGACCTTGTT
GGCTTTGTGGTGTGATAAAACACTCTCCATGGCCACTTGGCAGAGAGGCCAGCAGCCCGA
AGCAACTGTAATTAAAAGCCTGGCACTGAATGTTCCCTTTCCTTGTCATTGCACAAAATC
TGTGCTGCTTAGGTTAGGAGCAGAAGAAGGTGGGGAAGCTGGGGGGAGGGAAGACAAGAA
GGCAC

FIG. 1E

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 00/22986

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/85 C12N5/10 C07K14/705 C12Q1/68
A61K31/711 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, STRAND, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 875 566 A (SMITHKLINE BEECHAM CORP) 4 November 1998 (1998-11-04) page 12, line 13 - line 24; claim 6 ---	1-7
X	SAKURAI T ET AL: "Oxerins and oxerin receptors: A family of hypothalamic neuropeptides and G Protein-coupled receptors that regulate feeding behaviour" CELL,CELL PRESS, CAMBRIDGE, NA,US, vol. 92, 20 February 1998 (1998-02-20), pages 573-585, XP002105412 ISSN: 0092-8674 cited in the application the whole document --- -/--	1-7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2000	05/12/2000

Name and mailing address of the ISA	Authorized officer
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2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 00/22986

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	PEYRON CHRISTELLE ET AL: "A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains." NATURE MEDICINE, vol. 6, no. 9, September 2000 (2000-09), pages 991-997, XP002153570 ISSN: 1078-8956 the whole document	1-7
P,X	DATABASE GENBANK 'Online! Accession No. AL355514, 7 May 2000 (2000-05-07) PAVITT R.: "Human DNA sequence from clone RP11-73M7" XP002153572 the whole document	1-7
A	LIN LING ET AL: "The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene." CELL, vol. 98, no. 3, 6 August 1999 (1999-08-06), pages 365-376, XP002153571 ISSN: 0092-8674 cited in the application the whole document	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim 6 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claim 7 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No
PCT/US 00/22986

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0875566 A	04-11-1998	US 6020157 A	01-02-2000
		DE 875566 T	04-05-2000
		JP 10327888 A	15-12-1998
		JP 2000060578 A	29-02-2000